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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

Steroid hormones are involved in the development and growth of breast cancer. Drugs, which inhibit estrogen action, are commonly used to inhibit breast cancer growth. Unfortunately, most advanced breast cancer becomes resistant to estrogen treatment. Recently, many steroid receptor coactivators have been discovered and found to potentiate the transcriptional activity of steroid receptors and enhance the expression of hormone response genes. In the SRC-1 family of coactivators, AIB1 is found amplified and/or over-expressed in breast cancer specimens. To evaluate the potential roles of the SRC-1 family of coactivators in mammary tumorigenesis *in vivo*, we proposed to generate transgenic mice over-expression of AIB1 (SRC-3) in mammary glands. To target the expression of AIB1 in mammary gland, we placed the AIB1 transgene under the control of the MMTV-LTR promoter. Two lines of transgenic mice expressing AIB1 have been generated. Studies on these transgenic mice will help understand the development and progression of breast cancer and provide a molecular basis for designing novel strategies to curb and, ultimately, cure breast cancer.

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INTRODUCTION

Steroid hormones play a central role in mammary gland development. The hormonal effects are mediated by estrogen and progesterone receptors, which act as hormone dependent transcription factors . Upon ligand binding, these receptors bind to the cognate hormone responsive elements on the target promoter and recruit co-activators and general transcription factors to regulate the expression of hormone responsive genes. Several coactivators which interact with estrogen and progesterone receptors have been cloned and extensively characterized in various laboratories, including ours. One of the co-activator, Steroid Receptor Co-activator (SRC-1), has been demonstrated by us to possess intrinsic histone acetyltransferase (HAT) activity. This property may facilitate localized chromatin remodeling and enhance assembly of basal transcription factors, resulting in the increase of transcription of hormone response genes. In addition to SRC-1, two other related genes of the same family have recently been identified and characterized. These are TIF-2(GRIP-1/SRC-2) and AIB-1(p/CIP/ACTR/RAC-3 /TRAM/SRC-3). Like SRC-1, these two coactivators have also been demonstrated to possess HAT activity and to interact with members of the nuclear receptor superfamily to enhance their abilities to transactivate the target genes.

Two salient points strongly implicate members of the SRC-1 family of coactivators as potential oncogenes for mammary tumorigenesis. Firstly, our published results indicate that ablation of SRC-1 gene by homologous recombination in mice severely compromised mammary gland development in response to estrogen and progesterone. Secondly, AIB-1/SRC-3 is amplified in 10% of breast cancer patients and is over-expressed in 64% of primary breast cancer samples. Since these family of coactivators are essential for the maximal expression of hormone response genes, we hypothesize that ectopic over-expression of these genes may play a role in the initiation, progression or transition of tumor cell growth from a hormone dependent to a hormone independent state. To test our hypothesis, we will generate transgenic mouse lines over-expressing either SRC-1 or AIB-1/SRC-3 and assess whether over-expression of these genes will result in mammary gland hyperplasia. We expect the results obtained from these studies will address whether over-expression of this family of co-activators play a key role in breast tumor formation.

SIGNIFICANCE

AIB-1 is a member of the SRC-1 family of transcriptional coactivators which have been shown to be essential for the maximal activation of hormone responsive genes. This family of transcription factors, not only interacts with members of the large nuclear receptor superfamily, but also possesses HAT activity and interacts with other coactivators including CBP/P300 and P/CAF. Therefore, over-expression of AIB-1 in breast cancer cells may not only perturb the expression of many estrogen, progesterone and other hormonal regulated genes, but may also affect the signal tranduction pathways which depend on CBP/P300 and P/CAF. Therefore, the deregulated expression of AIB-1 or SRC-1 may have a profound effect on the growth and development of mammary gland. To investigate how members of the AIB-1 family influence

growth and development of mammary gland, we propose to use transgenic mice as a model system. Transgenic mouse lines over-expressing AIB-1 in the mammary gland will be generated. These lines will be used to assess whether over-expression of the AIB-1 will be sufficient to induce mammary tumorigenesis.

BODY

Expression of the AIB-1 in transgenic lines using MMTV-KCR-AIB-1 transgenic construct

Previously, we have obtained two transgenic founder lines, 2694 and 9845, expressing the AIB-1 in the mammary gland. However, the expression level of AIB-1 transgene is only 1 to 2 fold more than that of the endogenous mP/CIP (human AIB-1 homologoue). In an attempt to enhance the expression level of AIB-1 in the line 9845, a homologous line was generated from this founder line. RNA samples were isolated from transgenic mice in different developmental stages and the corresponding expression levels were detected by RNase protection assays using a AIB-1 specific riboprobe and normalized by the cyclophilin expression. In the virgin / non-pregnant transgenic mice, the expression level of AIB-1 transgene of homologous line 9845 (+/+) remained low and only 1 to 2 fold more than that of the endogenous mP/CIP. However, the expression level of AIB-1 was found to be increased at a much higher level during pregnancy (P6 and P17) and early lactation (L1) stages, and it was subsequently dropped back to the original level at the late lactation (Fig.1).

Assessment of the effect of over-expression of AIB-1 in mammary gland growth and development

Knowing that the homologous transgenic line 9845 expresses AIB-1 transgene at a higher level during pregnancy and early lactation, it is important to analyze for the physiological perturbation that may ascribe to the expression of AIB-1 in these stages. Mammary gland whole-mount at different stages of development, 6-month old virgin, pregnancy (day 6, P6; and day 16, P16) and lactation (day 1, L1; day 10, L10) were used for the assessment of the effect of over-expression of AIB-1 in mammary gland development. In the homozyous (+/+) transgenic mice whole-mount sections, the degree of mammary gland developments were similar to the age and stage-matched wild-type mice (Fig.2) and so far no obvious perturbation was found in the transgenic mammary gland.

As mentioned before, the expression of the transgene is pregnancy dependent. Thus, the multiparous transgenic glands have also been analyzed. As illustrated in Figure 2, both the wild-type and transgenic gland displayed similar phenotype. Only some of the mice showed aberrant morphology of squamous nodule in the ductal tree. Since similar phenotype has also been observed in the wild-type aged mice, we are currently assessing whether this is resulted from aging or from over-expression of AIB-1 (Fig.2).

Alternative approach to over-express AIB-1

It is possible that mammary epithelial cell might not tolerate high levels of AIB-1 expression, so we cannot over-express the AIB-1 transgene at a high level using the traditional constitutive expression method. To overcome this problem, an inducible system developed in our laboratory will be used. This system employs a transactivator and a target. The transactivator GLp65 is a chimic protein containing a mutated human progesterone receptor ligand binding domain fused with a yeast GAL4 DNA binding domain and a partial p65 protein activation domain. To generate the transactivator line, MMTV-KCR-GLp65, a transactivator was subcloned into a modified version of MMTV-KCR vector, which can drive a mammary gland specific expression. The KCR fragment contained the partial exon II, intron II, exon III and a endogenous polyadenylation signal derived from rabbit β globin gene. For the generation of the target, the AIB-1 transgene was placed under the control of four yeast transcription factor GAL4 binding sites (refereed as 17X4) and a TK promoter (Fig.3A). Crossing of the transactivator line with the target line can obtain a bitransgenic mice. With the administration of RU486, the regulator can then bind to the 17X4 UAS recognition sequences upstream of the target oncogene and induce the expression of AIB-1 transgene. The expression of AIB-1 in bitransgenic mice can be turned on by RU486 at a specific window during development and the effects of this coactivator on mammary gland oncogenesis can be monitored.

Characterization of the inducible system using FGF-3 as target

Previously, we have generated four transactivator lines. To elucidate the regulatory profile of the inducible system, the MMTV-transactivator line has been crossed with the FGF-3 target line to generate the bitransgenic mice. Different dosages of RU486 and different induction times have been applied on the bitransgenic mice and the corresponding expression profiles of transgene were examined as shown in Figure 3. The expression of the FGF-3 transgene in mammary gland can be tightly regulated by the administration of RU486 (Fig.3B). The kinetics analysis indicated that the FGF-3 RNA is accumulated up to a detectable level one day after administration of RU486 pellet and was maintained at a high level over weeks (Fig. 3C). In addition, the level of induced FGF-3 expression correlated with the dosage of RU486 administrated. A dose-dependent increase of FGF-3 RNA was detected when RU486 concentration was increased from 150 μg/kg to 450 μg/kg (Fig. 3D). In addition, we have also demonstrated that the long-term induction of FGF-3 results in mammary gland hyperplasia which can be reversed by RU486 withdrawal (Fig.4) Therefore, we believe that this inducible system is an useful animal model for our future study. The AIB-1 target line is now in preparation and will be crossed with this MMTV-transactivator line.

ONGOING EXPERIMENTS

Generation of AIB-1 target line

We have examined 7 AIB-1 target lines, but only one is potentially inducible. Thus, we are currently working on this line. By the way, more AIB-1 target lines will be generated. Once more lines are available, the transactivator line will be crossed with the target lines to get the bitransgenic mice and AIB-1 expression will be examined.

KEY RESEARCH ACCOMPLISHMENTS

1. Characterized AIB-1 expression profile of the homologous MMTV-AIB-1 transgenic line in different developmental stages.
2. Analyzed the mammary gland phenotypes of homologous transgenic mice expressing AIB-1 in different developmental stages.
3. Fully established and characterized the mammary gland specific inducible transgenic mice model using the FGF-3 target line.

REPORTABLE OUTCOMES

1. Completed the phenotypic analysis of the mammary gland of homologous transgnic mice constitutively expressing AIB-1.
2. Established and fully characterized an inducible system for over-expression of transgene in mammary gland.

CONCLUSION

In homologous AIB-1 transgenic line, we found that AIB-1 transgene is expressed at a high level during pregnancy and early lactation. Subsequent analysis using mammary gland whole-mounts at different developmental stages showed that there is no obvious phenotypic difference between transgenic mice and the age and stage-matched wild-type mice. The low expression of AIB-1 may account for the insignificant morphological changes. In an attempt to overexpress the AIB-1 to a high level as demonstrated in the cancer patient samples, we are currently generating the inducible system expressing the AIB-1 transgene under the control of RU486. Establishment of the inducible system will facilitate the analysis of the potential role of AIB-1 on the breast cancer formation and progression. It will also provide the molecular basis for design novel strategies to curb and ultimately cure breast cancer.

REFERENCES

NONE

APPENDICES

Fig. 1. **Expression profile of the AIB-1 transgene in transgenic mammary glands.** A) Ribonuclease protection assay of total RNA isolated from mammary glands at different developmental stages including V6M: virgin 6 month old; P6: pregnancy day 6; P16: pregnancy day 16; L1: lactation day 1; L10: lactation day 10. The expression of AIB-1 is increased during the pregnancy and early lactation.

Fig. 2. **Wholemount analyses of homologous (+/+) transgenic mice during various developmental stages.** Comparison of the transgenic mammary glands with the age and stage matched wild-type (WT). Transgenic mice displayed no obvious phenotypic perturbation during different developmental stages including V6M: virgin 6 month old; P6: pregnancy day 6; P16: pregnancy day 16; L1: lactation day 1; L10: lactation day 10. Multiparous transgenic mice also showed no significant difference from the wild-type.

Fig.3. **Generation and characterization of the RU486 inducible transgenic mice.** A) Schematic representation of the MMTV-KCR-GLp65 and AIB-1 traget. MMTV-KCR-GLp65: The transactivator GLp65 is a chimic protein containing a mutated human progesterone receptor ligand binding domain fused with a yeast GAL4 DNA binding domain and a partial p65 protein activation domain. It was placed under the control of MMTV-LTR promoter. AIB-1 traget: The AIB-1 gene was placed under the control of the rat collagenase TATA box and four Gal4 DNA binding sites (UAS). B) RU486 induced expression of FGF-3. The bigenic (Bi-Tg, GLp65 / UAS_G-FGF-3), monogenic target (UAS_G-FGF-3) or monogenic regulator (MMTV-KCR-GLp65) mice were subjected to the RU486 or the control vehicle (placebo) treatment at a dosage of 150 μg/kg of body weight for 2 weeks. Subsequently, the expression of the transactivator and the FGF-3 target gene were examined by RPA. C) The kinetics of the FGF-3 induction. The expression of the FGF-3 transgene in the bitransgenic mice (GLp65 / UAS_G-FGF-3) was examined in different time interval after the RU486 treatment with the dosage of 150 μg/kg of body weight. D) FGF-3 expression is dependent of the dosage of RU486. Increasing amount of RU486 was applied to the bitransgenic mice results in a dose dependent increase in the FGF-3 expression level. Consistently, no detectable FGF-3 expression was detected in the monogenic control treated with RU486.

Fig. 4. **Inducible expression of FGF-3 results in mammary gland hyperplasia.** Whole mount analyses of mammary glands were preformed from the control mongenic gland treated with 450 μg/kg RU486 for 4 months (Ctrl); bitransgenic mice treated with 450 μg/kg RU486 for 4 months (ON); and bitransgenic gland 2-month after withdrawal of RU486 (OFF). Lower panel: the RPA analyses indicates that the FGF-3 transgene can be turned on and off in the bitransgenic glands in response to the administration and withdrawal of RU486. No detectable FGF-3 was detected in the monogenic control in presence of RU486.

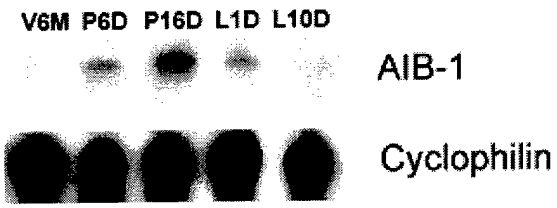
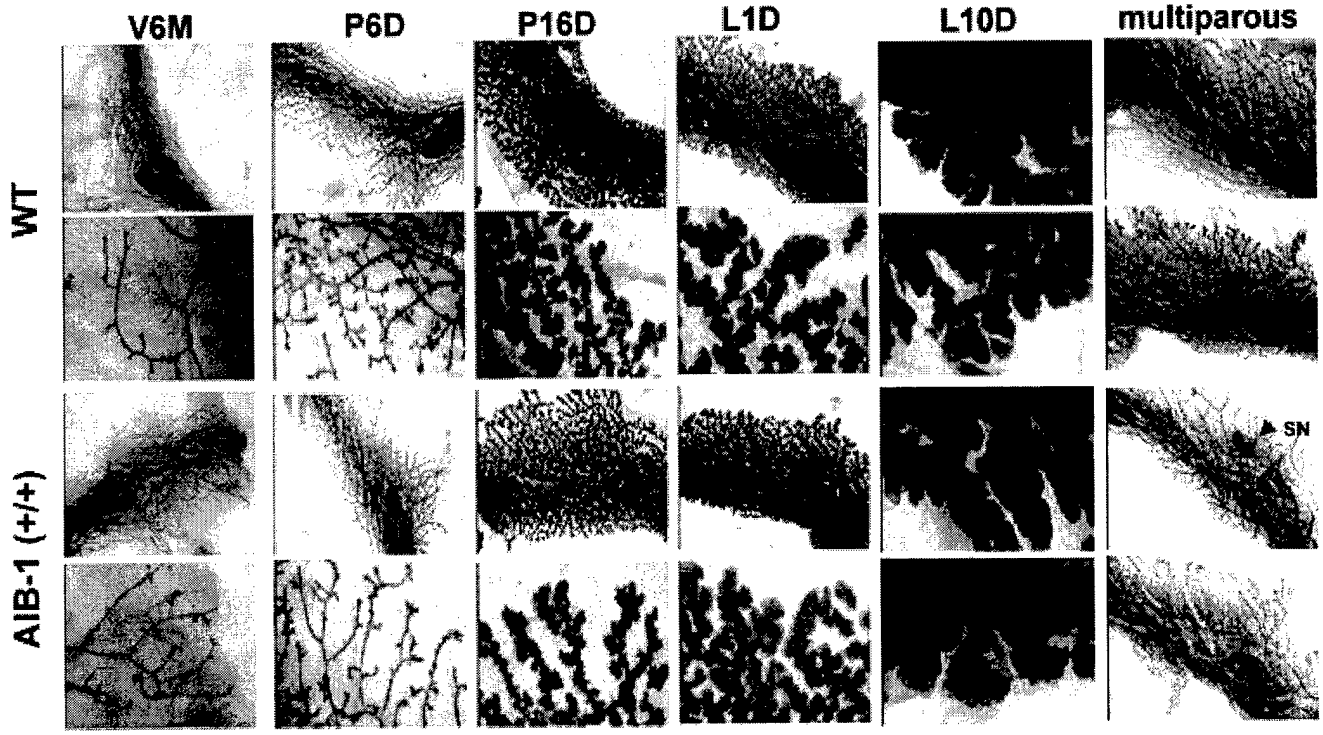
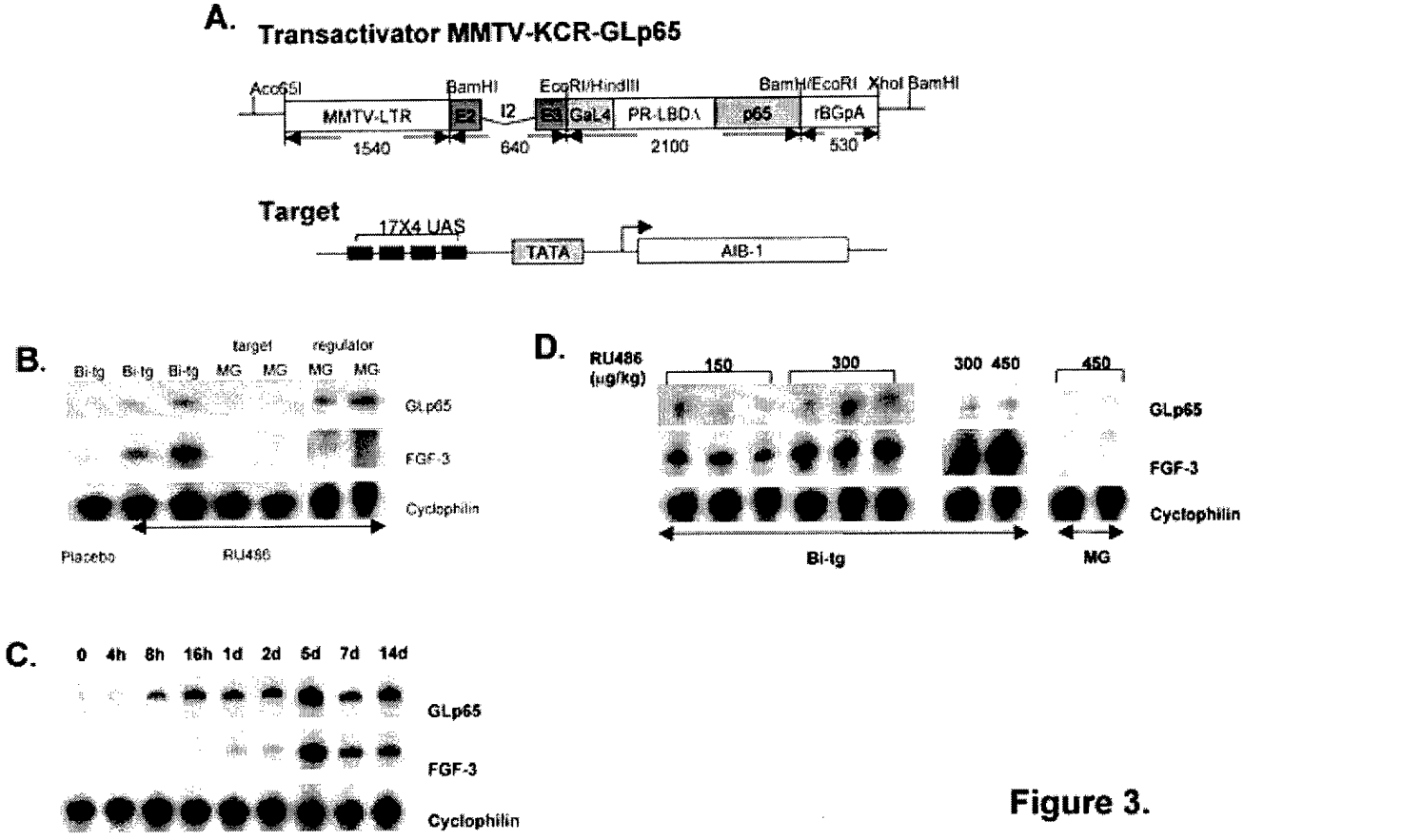


Figure 1.



SN : Squamous nodules

Figure 2.


A.
Transactivator MMTV-KCR-GLp65
Aco65I
BamHI
EcoRI/HindIII
BamHI/EcoRI
XhoI
BamHI
MMTV-LTR
E2
I2
E3
GaL4
PR-LBDΔ
p65
rBGpA
1540
640
2100
530
Target
17X4 UAS
TATA
AIB-1
B.
target
regulator
Bi-tg
Bi-tg
Bi-tg
MG
MG
MG
MG
GLp65
FGF-3
Cyclophilin
Placebo
RU486
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RU486
(µg/kg)
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FGF-3
Cyclophilin
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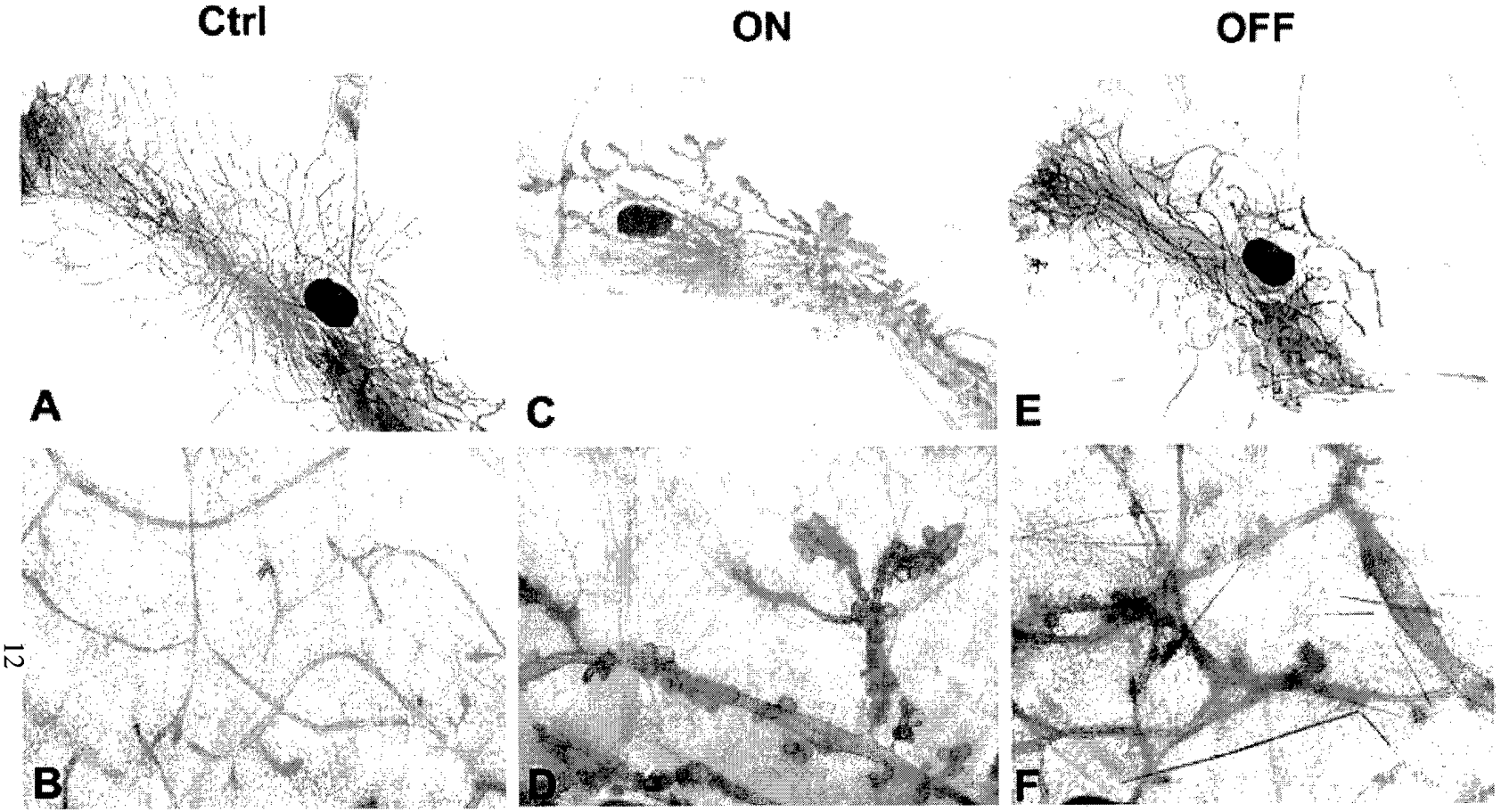
GLp65
FGF-3
Cyclophilin

Figure 3.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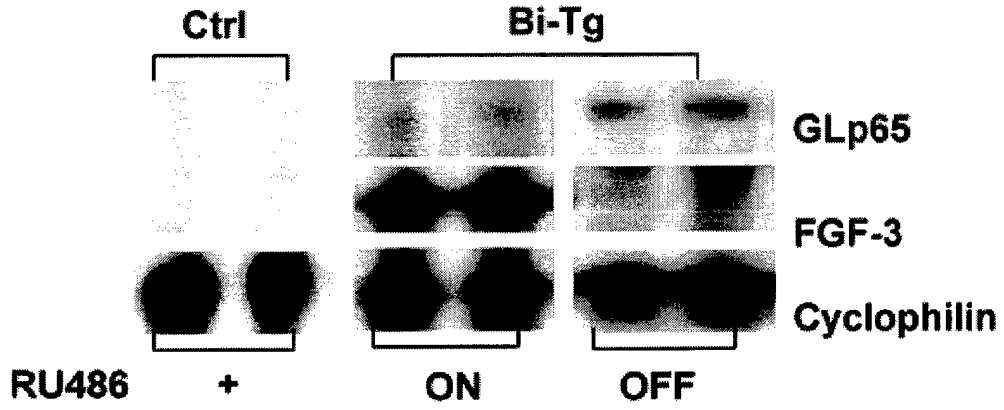
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